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Mediation of *Staphylococcus saprophyticus* Adherence to Uroepithelial Cells by Lipoteichoic Acid

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Treatment of uroepithelial cells with lipoteichoic acid from *Staphylococcus saprophyticus* resulted in a decrease in the adherence of this organism. Similar effects were observed when bacteria were pretreated with the lipoteichoic acid ligands albumin and anti-polyglycerophosphate monoclonal antibodies. Lipoteichoic acid might behave as an adhesin of *S. saprophyticus*.

Staphylococcus saprophyticus is a frequent cause of urinary tract infections, especially in young women (21). It has been proposed that the tropism of *S. saprophyticus* for the urinary tract can be explained by its greater ability to adhere to periurethral and uroepithelial cells (UC) than to epithelial cells from other body areas (5). Only a few studies have focused on the molecular basis of *S. saprophyticus* adherence. Surface charge and hydrophobic interactions may be involved (4). Recently, the agglutination of sheep erythrocytes by *S. saprophyticus* was shown to be mediated by heat- and protease-sensitive bacterial adhesins (7) and by specific disaccharide elements on the erythrocyte membrane (6). In the present study we have examined the possible role of lipoteichoic acid (LTA) in mediating the attachment of *S. saprophyticus* to UC. LTAs are composed of a lipid portion covalently linked to a polyglycerophosphate chain and are believed to be the adhesins of *Streptococcus pyogenes* (15), *Streptococcus agalactiae* (14, 20), and *Staphylococcus aureus* (1).

S. saprophyticus GS373 and GT222 and *Escherichia coli* 238 were clinical urinary isolates. *Staphylococcus* strains were identified by the method of Kloos and Schleifer (9). Monoclonal anti-polyglycerophosphate antibody (MAb) 7B11 (8), a generous gift of G. D. Shockman, Temple University School of Medicine, Philadelphia, Pa., was purified from ascites fluid by affinity chromatography on protein A-Sepharose CL4B (Pharmacia Fine Chemicals, Uppsala, Sweden) as described previously (17). Human serum albumin and keyhole limpet hemocyanin were purchased from Calbiochem, San Diego, Calif. Glycerol, phosphorus, and protein in bacterial extracts were assayed by the methods of Chernick (3), Chen et al. (2), and Lowry et al. (10), respectively. Nucleic acid content was estimated photometrically at 260 nm. Alanine was determined quantitatively with an automated amino acid analyzer (Carlo Erba, Milan, Italy) after hydrolysis in 2 M HCl at 100°C for 150 min.

S. saprophyticus LTA was purified as outlined below and deacylated by ammonia hydrolysis as described by Ofek et al. (15). The LTA content of different preparations was estimated by passive hemagglutination (13, 15) and expressed as the minimum concentration needed to sensitize sheep erythrocytes, or sensitizing activity. Hemagglutination tests were performed with purified MAb 7B11 at a concentration of 30 μg/ml or MAb-containing ascites fluid at a 1:100 dilution. Inhibition of hemagglutination by deacylated LTA was performed to demonstrate that ammonia hydrolysis had indeed resulted in deacylation of LTA without affecting the integrity of the polyglycerophosphate backbone (15). Deacylated LTA prevented MAb-mediated agglutination of erythrocytes sensitized with LTA (50 μg/ml) at a minimal concentration of 10 μg/ml.

Adherence tests were performed as previously described, with minor modifications (20). Briefly, bacteria grown to the early stationary phase in tryptic soy broth (Difco Laboratories, Detroit, Mich.) were mixed with UC collected from pooled clinical urine specimens at a 5,000:1 ratio and incubated at 37°C for 30 min in 1 ml of phosphate-buffered saline (0.02 M phosphate, 0.15 M NaCl, pH 7.2) (PBS). The above conditions were found to be optimal in preliminary experiments.

In particular, adherence was maximal over a broad pH range (5.5 to 8.0) and at the late logarithmic and early stationary phases of bacterial growth (data not shown). Bacteria-to-UC ratios greater than 5,000:1 resulted in unacceptable (>10 cocci per microscopic field at ×1,000 magnification) background levels when determining adherence (see below). In pretreatment experiments, bacteria or UC were incubated at 37°C for 30 min with the indicated substances or with plain PBS, washed twice in PBS, and suspended to the appropriate concentrations before adherence testing. Unbound bacteria were removed by filtration through polycarbonate filters (8-μm pore size; Bio-Rad Laboratories, Richmond, Calif.). The total number of bacteria associated with 40 UC was determined by microscopic observation of the filters. Only UC pools that did not show naturally adherent bacteria were used. In preliminary experiments adherence determinations showed satisfactory intraexperimental reproducibility, although considerable variation was sometimes observed with different pools of UC, as can be seen in the controls reported in the legends of Fig. 2 and 3. Assays of agglutination and agglutination inhibition of sheep erythrocytes by *S. saprophyticus* were performed with microtiter plates (6, 7). Bacterial hydrophobicity was assayed as described previously (16), with minor modifications, by quantitating adherence to hexadecane droplets.

For extraction of LTA, strain GS373 was grown to the early stationary phase at 37°C in 2-liter batches of tryptic soy broth. The cells were harvested by centrifugation at 8,000 × *g* for 15 min, washed twice with PBS, and extracted with aqueous phenol (46%, vol/vol) for 30 min at 60°C as described by Moskowitz (12). The aqueous phase was dialyzed

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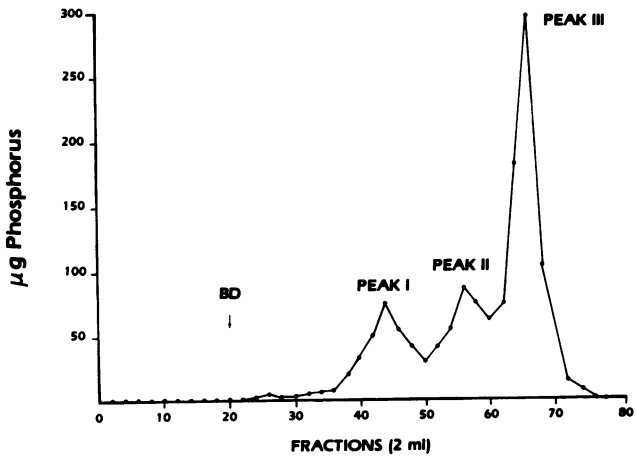

FIG. 1. Elution profile of a crude phenol extract from *S. saprophyticus* cells. The material (121 mg, containing 2.3 mg of phosphorus) was eluted from a column of Sepharose 6B (1.5 by 87 cm) with 0.2 M ammonium acetate at a flow rate of 4.5 ml/h, and alternate fractions were assayed for phosphorus content. BD, Blue dextran.

against distilled water and lyophilized. The crude extract was loaded on a Sepharose 6B column (1.5 by 87 cm; Pharmacia) and eluted with 0.2 M ammonium acetate. Fractions within each of three phosphorus-containing peaks (Fig. 1) were pooled, dialyzed against distilled water, and lyophilized.

Erythrocyte-sensitizing activity (31.2 μg/ml) was detected only in peak 1. Protein and nucleic acid accounted for 9.9 and 4.8%, respectively, of the dry weight of this material. Further purification of LTA in peak 1 was attempted by affinity chromatography with human albumin immobilized on agarose as described by Simpson et al. (18). Briefly, albumin was coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia) as specified by the manufacturer. In a typical experiment, peak 1 material (7.7 mg) was introduced into a column (0.9 by 12 cm) of albumin-Sepharose in PBS, allowed to equilibrate, and sequentially eluted with PBS and with PBS containing 50% ethanol. The PBS and PBS-ethanol fractions were dialyzed against distilled water and lyophilized. About 88% of the applied material was recovered in the two fractions. Material eluted with PBS-ethanol (5.3 mg) had protein and nucleic acid contents of <1 and 0.8%, respectively. Sensitizing activity was 15.6 μg/ml.

This material was considered to represent purified LTA because phosphorus and glycerol were present in equimolar amounts and glycerophosphate accounted for 83% of the weight. The LTA molecule contained an average of 25 residues of glycerophosphate, as calculated from the ratio of organic to inorganic phosphorus after terminal phosphate hydrolysis with alkaline phosphatase (19). On the basis of quantitative amino acid analysis, the polyglycerophosphate chain was considered to be substituted with 1 D-alanine per 3.4 glycerophosphate units. Binding of *S. saprophyticus* LTA to albumin was confirmed by showing inhibition of heat coagulation of albumin by LTA (Table 1). These results confirm those obtained by Simpson et al. with streptococcal LTA (18).

Pretreatment of UC with 100 μg of LTA, but not with deacylated LTA, caused 67.9 and 66.8% inhibition in the adherence of strains GS373 and GT222, respectively (Fig. 2). Adherence inhibition was dose dependent and saturable.

TABLE 1. Inhibition of heat coagulation of albumin by *S. saprophyticus* LTA

Agent added to albumin	Albumin coagulated[a]	
	μg	%
None	3.360	84.0
Deacylated LTA (100 μg)	3.130	78.3
LTA (100 μg)	550	13.8

[a] Human albumin (4 mg in 2 ml of H_2O) was heated for 30 min at 80°C. Aggregated albumin was separated by centrifugation at 15,000 × *g* for 20 min, and the supernatant was analyzed for protein. Coagulated albumin was calculated from the loss of soluble albumin after heating.

The adherence of *E. coli* 238 was only marginally affected by LTA (Fig. 2). To confirm the possible role of LTA as an adhesin, in further experiments *S. saprophyticus* GS373 was treated with two substances known to bind LTA, such as MAb 7B11 (8) and human serum albumin (18) before adherence testing. Both of these substances had inhibitory effects (Fig. 3). Pretreatment with MAb 7B11 resulted in significant inhibition at concentrations ranging from 1.25 to 5 μg/ml (Fig. 3). Inconsistent results were obtained with higher concentrations, probably because of the formation of microscopically visible aggregates. Human albumin also appeared to prevent adhesion in a dose-related fashion, with maximal inhibition (76.1%) at 125 μg/ml (Fig. 3). Keyhole limpet hemocyanin, on the other hand, did not affect adherence at doses up to 500 μg/ml, thus excluding nonspecific protein effects.

Since some *S. saprophyticus* strains are known to cause agglutination of sheep erythrocytes (6), it was of interest to investigate whether LTA or LTA ligands inhibit this phenomenon. Hemagglutination by strain GT222 was not inhibited by LTA (500 μg/ml), albumin (500 μg/ml), or MAb 7B11 (30 μg/ml), while strain GS373 did not induce hemagglutination (data not shown).

Results presented here suggest a role for the lipid portion of LTA in the adherence of *S. saprophyticus* to UC. LTA might favor adherence by combining to specific receptors or by increasing surface hydrophobicity (11). Thus, it was of interest to ascertain whether the LTA ligands shown above

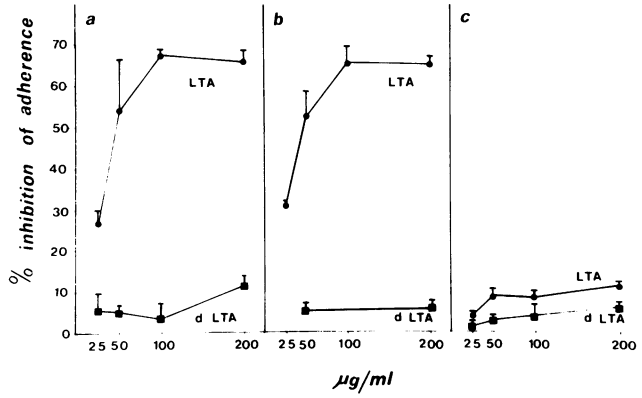

FIG. 2. Effect of pretreatment of UC with various concentrations of LTA or deacylated LTA (dLTA) on the adherence of *S. saprophyticus* GS373 (a), *S. saprophyticus* GT222 (b), and *E. coli* 238 (c). Points and bars represent means ± standard deviations for three experiments. The numbers of bacteria adhering to 40 UC in control experiments were 254 ± 117 (*S. saprophyticus* GS373), 289 ± 138 (*S. saprophyticus* GT222), and 704 ± 176 (*E. coli* 238).

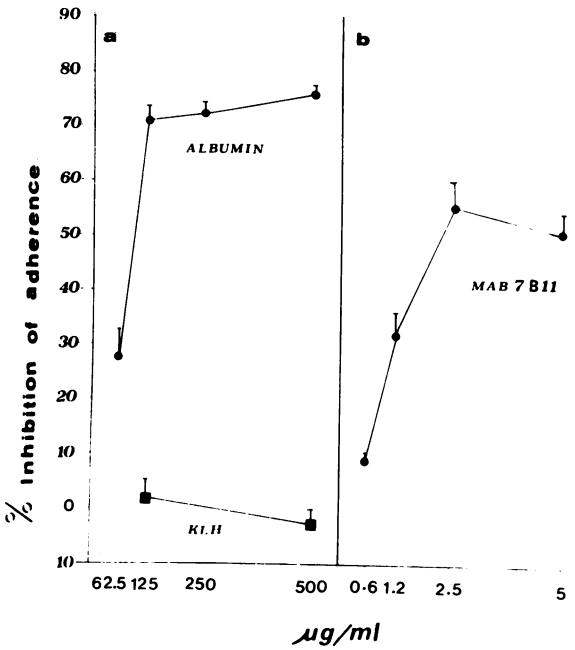

FIG. 3. Effect of pretreatment of *S. saprophyticus* GS373 with various concentrations of albumin (a) and anti-polyglycerophosphate MAb 7B11 (b) on adherence to UC. Points and bars represent means ± standard deviations for three experiments. The numbers of bacteria adhering to 40 UC in control experiments were 134 ± 53 (a) and 154 ± 81 (b). KLH, Keyhole limpet hemacyanin.

to inhibit adherence were also capable of affecting bacterial hydrophobicity.

Treatment of both *S. saprophyticus* strains with MAb 7B11 or albumin followed by washing did not affect their adherence to hexadecane, (Table 2), and therefore this possibility was unlikely. As a corollary it should be noted that the adherence to UC of these two strains was similar in extent and sensitivity to LTA (Fig. 2), although their adherence to hexadecane was quite different (Table 2). Clearly, the role of *S. saprophyticus* LTA in determining surface hydrophobicity and the relationship of the latter to adherence to UC need further investigation.

Although our observations indicate that LTA might behave as an adhesin of *S. saprophyticus*, they do not exclude the possibility that molecules other than LTA might also mediate or modulate the adherence process. It should be noted that adherence was never totally abolished by the LTA-specific treatments tested here. In this respect the observation that agglutination of sheep erythrocytes by *S. saprophyticus* depends on binding to terminal β-D-galactose-*p*-(1-4)-β-D-2-acetoamido-2-deoxyglucose-*p*-(1- residues on erythrocyte membranes (6) might be of relevance also for adherence to human UC. However, based on agglutination inhibition experiments, this type of interaction seems quite distinct from LTA-mediated adherence.

TABLE 2. Effect of albumin and MAb 7B11 on adherence to hexadecane of *S. saprophyticus* strains

Strain	Treatment[a] (μg/ml)	% Adherence to hexadecane[b]
GT222	None (PBS)	31.2
	Albumin (500)	27.7
	MAb 7B11 (5)	29.1
	KLH (500)	27.1
GS373	None (PBS)	78.4
	Albumin (500)	75.4
	MAb 7B11 (5)	74.6
	KLH (500)	73.5

[a] Bacteria were incubated with the indicated substances for 30 min at 37°C, washed two times, and suspended in PBS (A_{560} = 1.0) for the adherence to hexadecane assay. KLH, Keyhole limpet hemacyanin.

[b] Hexadecane (0.3 ml) was added to 1.2 ml of bacterial suspension and vortexed for 1 min. Adherence to hexadecane was expressed as the percent decrease in A_{560} of the aqueous phase after mixing with hexadecane.

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